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Imaging and clinical outcome of non-small cell lung cancer

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Kerner, G. S. M. A. (2016). *Imaging and clinical outcome of non-small cell lung cancer*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen.

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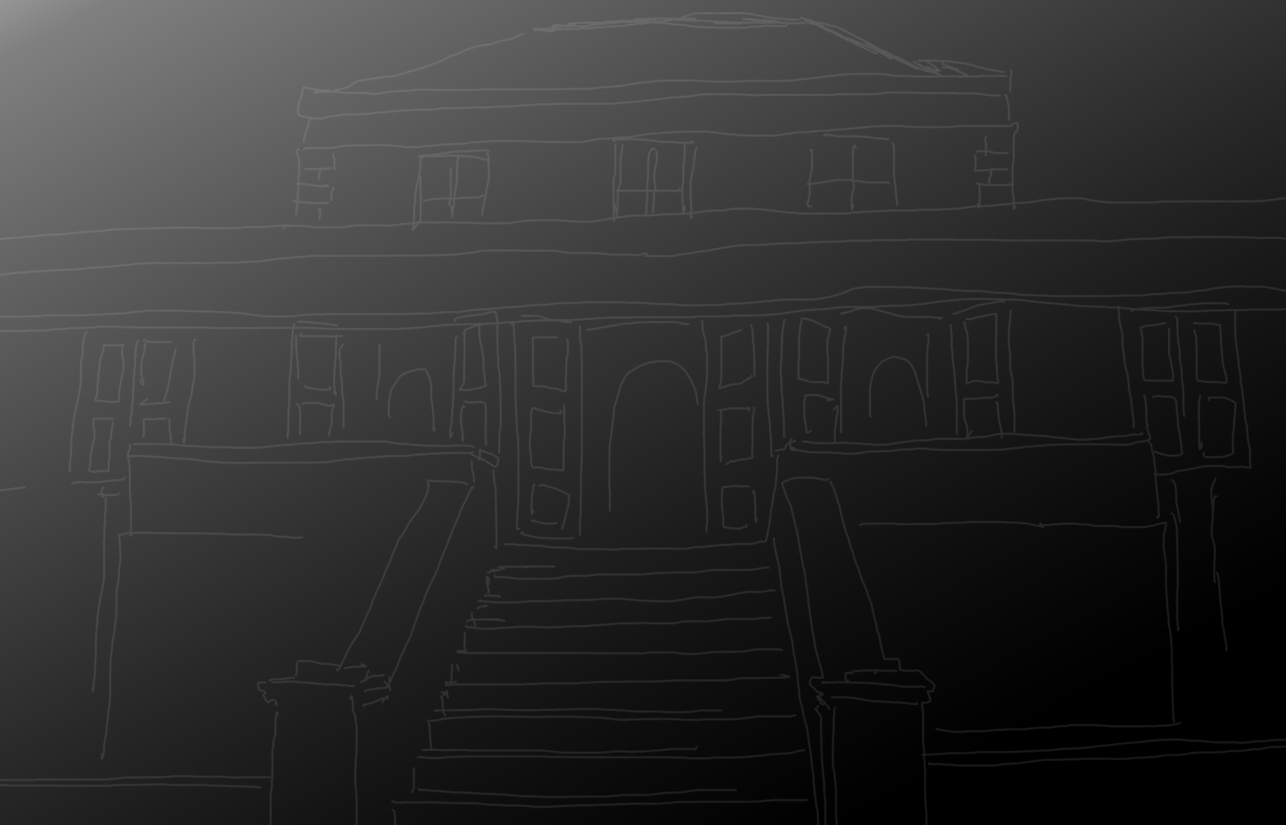
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NORMAL TISSUE METABOLISM DURING TREATMENT WITH PHOSPHOINOSITIDE-3- KINASE INHIBITOR AND CHEMOTHERAPY IN NON-SMALL CELL LUNG CANCER PATIENTS

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Submitted



Abstract

Phosphoinositide 3-kinases (PI3K) are enzymes involved in tumor growth, proliferation, motility and survival. In normal tissue, PI3K cooperates with insulin growth factor and the insulin signaling pathway. Pictilisib (GDC-0941) is a PI3K inhibitor with effects on glucose metabolism in normal and tumor tissue. Pictilisib combined with chemotherapy was studied in NSCLC patients to assess whether it induces metabolic changes in normal and tumor tissue as measured with ^{18}F -FDG PET/CT.

Methods

Patients were treated with pictilisib during 2 weeks each cycle combined with chemotherapy administered every 3 weeks. ^{18}F -FDG PET/CT was performed prior to and 2 weeks after start of cycle 2 and 4. In this exploratory pilot study, changes in metabolic activity (SUV_{max} and SUV_{mean}) in normal hepatic, muscular and cerebral tissue was studied by assessing ^{18}F -FDG activity. Metabolic tumor response was measured according to the 1999 EORTC recommendations whereas tumor response was measured according to RECIST 1.1 criteria. Measure of agreement was assessed using the McNemar test.

Results

In 14 patients ^{18}F -FDG uptake in hepatic, muscular and cerebral tissue did not change compared to baseline, except for a slight decrease in hepatic ^{18}F -FDG uptake at cycle 4. At the same time ^{18}F -FDG uptake in the tumor decreased and in patients with small scattered solid tumor parts PET showed large variations in ^{18}F -FDG uptake at the last day of pictilisib administration.

Conclusion

Pictilisib did not show clinically relevant metabolic changes in normal tissues when used with chemotherapy under fasting conditions.

Keywords: *NSCLC, pictilisib, PI3K, ^{18}F -FDG, PET/CT*

Introduction

Phosphoinositide 3-kinases (PI3K) are enzymes that regulate glucose uptake via the glucose transporter (GLUT)-4 receptor¹. The PI3K/Akt pathway is therefore integral to cellular glucose metabolism in both tumor and in normal tissue. Many distinct pathways converge to activate PI3K, giving the PI3K/Akt pathway a central role inside the normal cell.

In vitro tumor models have shown that constitutive PI3K activation results in insensitivity to both insulin and insulin-like growth factor-1 (IGF-1) signaling². Besides the insulin receptor, many other kinases target/activate the PI3K/Akt pathway. These kinases include epidermal growth factor receptor (EGFR) and Kirsten Rat Sarcoma viral oncogene (KRAS).

In non-squamous lung cancer, mutations in the oncogenes that code EGFR (10-20%) and KRAS proteins (20-30%) are frequent³⁻⁶. In squamous cell lung cancer, PI3K activity itself is frequently altered either due to mutations (9%) or due to amplifications (37%) of the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), also known as the p110 α protein oncogene^{7,8}. Finally, the metabolic signaling of PI3K pathway is influenced by the tumor suppressor “phosphatase and tensin homologue” (PTEN). PTEN acts as the most important negative regulator of the PI3K signaling pathway⁹. PTEN haploinsufficiency is linked to insulin sensitization that results in obesity with a decreased risk of type 2 diabetes¹⁰. Therefore, because of the central metabolic role of PI3K due to either EGFR/KRAS mutations which activate PI3K, or due to PI3K activity alterations, PI3K is an attractive target for inhibition of tumor growth in NSCLC patients. However, as PI3K is also part of an important pathway in the normal tissue, inhibiting PI3K could (negatively) affect glucose uptake in normal tissue. Supporting this theory is the observation that hyperglycemia was frequently noted during investigational trials¹¹.

One such PI3K inhibitor is pictilisib (GDC-0941), which is a highly selective inhibitor of the p110 α protein with little activity against mammalian target of rapamycin (mTOR). A previous phase 1 study of pictilisib was performed, in which patients with various types of cancer (excluding NSCLC) were treated, which established a safe and tolerable dose¹².

^{18}F -FDG PET/CT is a technique that allows the in vivo quantification of glucose metabolism. It is used in clinical practice for staging and tumor response assessment in non-small-cell lung cancer NSCLC patients. Although the uptake of ^{18}F -FDG is mainly dependent on GLUT-1 and GLUT-3 expression¹³⁻¹⁵, during PI3K inhibition ^{18}F -FDG might be a suitable marker to measure tumor response, as has been shown in a 3D tumor spheroid cell line model¹⁶. The same study showed that ^{18}F -FDG uptake is dose-dependent¹⁶.

The primary goal of this exploratory pilot study is whether PI3K inhibition with pictilisib (combined with chemotherapy) has a measurable effect on glucose metabolism in normal tissue (muscle, liver and brain) of advanced NSCLC patients.

Methods

Patients

Patients with advanced NSCLC were treated with chemotherapy. Patients with untreated known cerebral metastases were excluded from this study. Additionally, during the first 2 weeks of each 21 days cycle patients received pictilisib 260 mg orally once daily. All patients received standard anti-emetics, including dexamethasone, on day -1 and days 1, 2 and 3 of each chemotherapy cycle.

^{18}F -FDG PET/CT

^{18}F -FDG PET/CT scans were made at baseline, on day 14 of the second cycle and on day 14 of the fourth cycle of treatment on the last day of pictilisib administration. Times were chosen to assess the effect of pictilisib on ^{18}F -FDG metabolism optimally. ^{18}F -FDG-PET/CT scans were made on a Siemens Biograph mCT scanner. The image acquisition procedures and reconstructions were performed according to EANM guidelines¹⁷ with a volumetric voxel size of 4 by 4 by 2.4 mm (38.4 mm³). Prior to tracer injection, appropriate fasting blood glucose level after a 6 hour fasting period was confirmed and recorded and should be below 11 mmol/l. Patients were injected with ^{18}F -FDG dosed at 3 MBq/kg bodyweight intravenously. After 60 minutes, the PET scan was made. Scan times per bed position are depending on patients weight. If less than 60 kg, scan time was 1 minute, between 60-90 kg 2 minutes and above 90 kg 3 minutes per bed position¹⁸. The patients were scanned from the upper leg to the brain.

Contrast enhanced CT was performed on the Somatom CT, which is part of the mCT scanner, in the same session. Scan time was performed in 8 seconds, craniocaudally at inspiration. Effective mAs was 80, 120 kV with the care dose setting active. Slice thickness was 0.5 mm, pitch was 1.4 with a rotation of 0.5 seconds. Patients were injected with 55 ml of Iomeron contrast 350 mg/ml (Bracco Imaging Deutschland GmbH, Konstanz, Germany) at a speed of 2.5 ml/sec.

Image analysis of normal tissue

All PET related measurements were performed using an IMALYTICS Research Workstation (Philips Technologie GmbH Innovative Technologie, Aachen, Germany). In order to assess the effect of pictilisib on normal tissue, volumes of interests (VOIs) were drawn in muscular tissue, inside the brain, and inside the liver. The VOI used to assess muscular activity was drawn in the left gluteus maximus muscle, the volume needed to be at least 40 mL and the image was drawn using the low dose CT. The VOI inside the liver was at least 100 mL and carefully avoiding liver metastases. An experienced nuclear physician checked the VOI in patients with liver metastases to assure that the VOI was properly drawn. The VOI in the brain was drawn automatically using an adaptive threshold technique. No cerebral mean standardized uptake value (SUV_{mean}) was recorded. For the other VOI maximum standardized uptake value (SUV_{max}) and SUV_{mean} were calculated and recorded. No partial volume correction was used for normal tissue measurements.

Tumor measurements

The tumor size of the primary tumor and response was measured on CT according to RECIST 1.1 criteria¹⁹. This measurement was performed by an independent experienced radiologist.

On ^{18}F -FDG PET/CT the primary tumor lesions were defined using the adaptive threshold technique at 41% of SUV_{max} based on the study by Cheebsumon et al²⁰. Subsequently, correction for partial volume effect (PVE) was applied to the SUV_{max} value if the shortest axis of the largest diameter of the lesion was less than 28 mm as described previously²¹. Tumor lesions measuring less than 10 mm in the shortest axis were excluded from SUV_{max} further analysis, as were radiological appearances of less solid parts (e.g. adenocarcinoma in situ) on CT. Tumor ^{18}F -FDG response to treatment was defined according to the 1999 EORTC recommendations²².

Statistics

All standardized uptake values (SUVs) were normalized to a blood glucose level of 5.0 mmol/l ($SUV = SUV_{\text{measured}} * (\text{actual glucose}/5.0)$). ¹⁸F-FDG uptake in normal tissue and the primary tumor was compared between baseline and after 2 and 4 cycles respectively, using the Wilcoxon signed rank test. The agreement in response between CT and ¹⁸F-FDG PET/CT response classification was performed using the McNemar test. Whether the timing of the PET/CT (whether the scan was performed within the 2 week pictilisib treatment or during the stop week) had an effect on response measurement was assessed using the Kurskal-Wallis test. A p-value of <0.05 was considered significant. All statistics were performed using SPSS 22.0 (International Business Machines Corp, Armonk, NY, USA).

Results

Patient characteristics

Fourteen patients were included, 4 males and 10 females (Table 1). All patients were Caucasians and had advanced NSCLC.

Table 1: Patient characteristics

Number of patients	14
Age	58 (40-79)
Sex male/female	04/10
Histology:	
Adenocarcinoma	13
Squamous cell carcinoma	1

¹⁸F-FDG uptake in normal tissue

Fasting serum glucose levels were similar between baseline, after 2 cycles (P=0.90). There was a slight increase after 4 cycles of therapy (P=0.05) with 1 patient having developed hyperglycemia (Fig. 1A). After 4 cycles of chemotherapy with pictilisib the mean (sd) hepatic uptake of ¹⁸F-FDG using SUV_{max} was 3.4 (0.8) compared to 3.8 (1.1) at baseline (p <0.01) (figure 1B). No differences were observed in the cerebral or muscular glucose uptake during pictilisib administration between baseline, and after 2 and 4 cycles, respectively (Figure 1C-D).

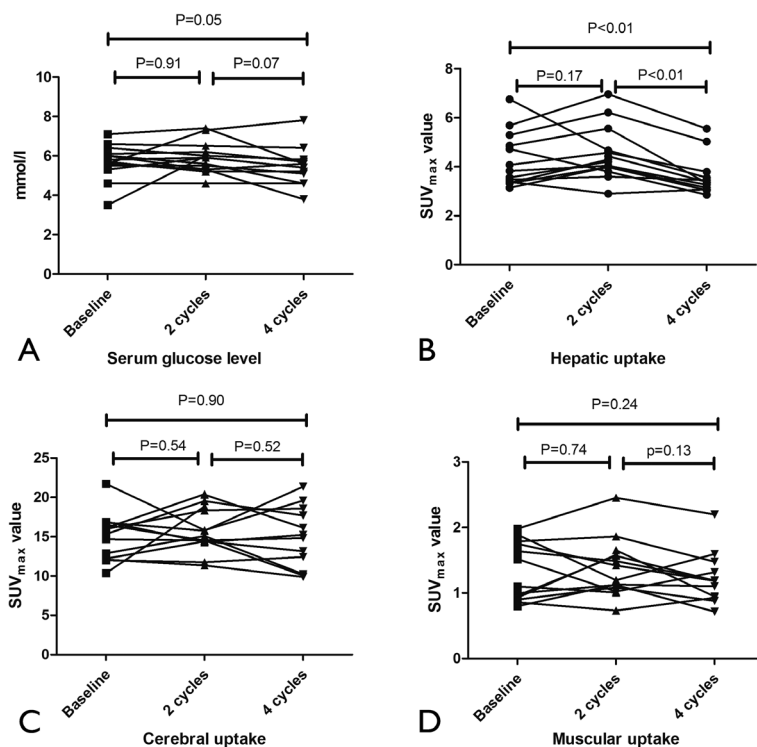


Figure 1: Physiologic metabolism assessments before, after 2 and 4 cycles with pictilisib and chemotherapy.

No change in serum glucose was seen. A small ^{18}F -FDG uptake change in hepatic tissue (b) was observed. No changes were evident either in cerebral tissue (c) or muscle (d).

Tumor measurements

In the 14 patients treated with pictilisib, 7 patients had a partial response and 7 had stable disease. Median (range) baseline SUV_{max} was 13 (10-28). At cycle 2 measured at the time of the last pictilisib administration at day 14, median SUV_{max} was 9 (5-16), and after 4 cycles also measured at the time of the last pictilisib administration at day 14, it was 6 (4-12) ($P=0.05$) (figure 2). None of these patients had metabolic progressive disease at cycle 4. An example maximum projection image of a patient at baseline and after 2 cycles is included (fig. 3A and 3B, respectively). ^{18}F -FDG PET/CT showed 7/10 metabolic partial responders at cycle 2 and 3/10 stable metabolic disease measured at the time of the last pictilisib administration at day 14. In 4 patients with scattered primary tumors SUV_{max} measurement was assessed without PVE correction showing increased ^{18}F -FDG uptake.

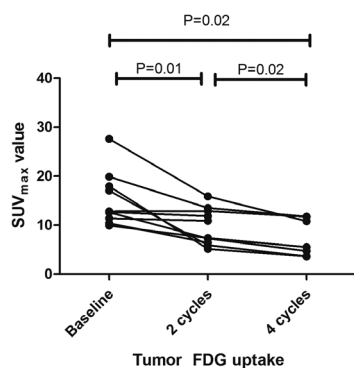


Figure 2: ^{18}F -FDG uptake in the primary tumor before and during chemotherapy and pictilisib.

Discussion

In this exploratory pilot study the metabolic effects of PI3K inhibition in combination with standard chemotherapy were assessed with ^{18}F -FDG PET/CT in patients with advanced NSCLC. The addition of PI3K inhibition did not result in a clinically relevant decline in ^{18}F -FDG uptake in normal tissue.

PI3K inhibition and glucose uptake in normal tissue

Hyperglycemia is sometimes observed during PI3K inhibition¹¹. In our fasting patients only in 1 patient hyperglycemia was observed at the day of the ^{18}F -FDG PET/CT. ^{18}F -FDG uptake in visceral organs has been previously used in different diabetes treatment related studies involving cerebral²³, hepatic²⁴ and muscular²⁵ ^{18}F -FDG PET uptake. These studies showed that various types of diabetes treatment influenced localized ^{18}F -FDG uptake compared to placebo²³⁻²⁵. It should be noted that in our study, we observed slight significant changes in glucose level. This confirms the results of Sarker et al, who found small (+/- 1.1 fold increase) yet significant changes in plasma glucose level at the dose of 330-340 mg pictilisib¹². Importantly, a study by Aggarwal et al found that Caucasians less often showed metabolic side effects of PI3K inhibition compared to Asians²⁶.

Pictilisib may exert its glucose uptake effects by PI3K inhibition through inhibition of the membrane GLUT-4 receptor¹. GLUT-4 is most abundantly expressed in cardiac, skeletal muscle and adipose tissue and is very important for insulin

stimulated glucose uptake^{27, 28}. GLUT-4 is also found in the brain, kidney and in the intestines²⁸. Consequently, one will expect uptake changes of ^{18}F -FDG in normal human tissues. How PI3K influences GLUT-1 is still disputed^{29, 30}. One study describes ^{18}F -FDG uptake mediated through PI3K signaling in endothelial cells through fibronectin³¹.

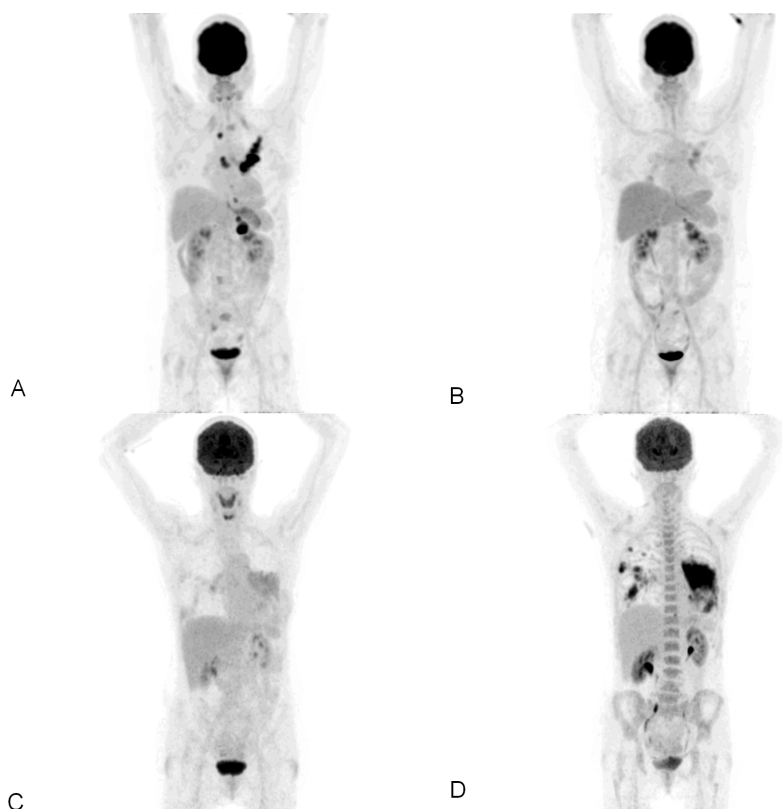


Figure 3: Maximum intensity projection at baseline and after 2 cycles of pictilisib and chemotherapy.

Baseline image of patient with solid NSCLC (A) and patient with less solid parts NSCLC (C). Image made after 2 cycles of therapy in patient 1 (B) and patient 2 (D). Patient 1 had a clear decrease in uptake within the primary tumor and metastasis, consistent with a partial metabolic response according to the 1999 EORTC recommendations, and had a PFS 7.6 months. Meanwhile, the uptake within the both the tumor and metastasis of patient 2 increased substantially, consistent with progressive metabolic disease. The PFS of patient 2 was 23.3 months.

Surprisingly, despite the frequent observations in prior studies of hyperglycemia during PI3K inhibition, in normal tissue PI3K inhibition by pictilisib caused no changes. In the liver only a small, statistically significant but probably clinically

irrelevant change in ^{18}F -FDG uptake was observed. GLUT-4 physiology may explain these findings. GLUT-4 is prevalent intracellularly, yet direct stimulation (either by insulin or muscle contraction induced) is necessary in order to translocate GLUT-4 from the intracellular storage vesicle to the plasma membrane to start taking up glucose^{27, 28}. Theoretically, fasting and resting between ^{18}F -FDG injection and the PET/CT, as per protocol is required when performing PET/CT, negates all factors necessary for GLUT-4 stimulation. During fasting no direct stimulation of GLUT-4 activity occurs. Our results suggests that PI3K inhibition by pictilisib does not influence GLUT-4 stimulation under fasting conditions. Simulating normal transient activity is necessary in order to definitively answer the question whether PI3K inhibition has any clinically relevant effects on normal tissue. One way to do this is to measure insulin sensitivity using the hyperinsulinemic euglycemic clamp technique prior to and after administration of a PI3K inhibitor.

PI3K inhibition and glucose uptake in tumor tissue

There is little literature concerning the clinical value of measuring ^{18}F -FDG uptake during PI3K inhibition. One paper described in a 3D spheroid cell line model that ^{18}F -FDG may be a suitable marker of response¹⁶. An animal study showed that ^{18}F -FDG uptake in mice with ovarian cancer treated with a PI3K/mTOR inhibitor was correlated to a decrease in cell proliferation³². Sarker et al investigated PI3K inhibition with pictilisib in their phase 1 study. In 32 patients, they included ^{18}F -FDG PET response assessment, and in 7 of these 32 (22%) patients had a metabolic partial response¹². In resected NSCLC, there is a direct relationship between ^{18}F -FDG uptake and the expression of glucose transport receptors (GLUT-1 and GLUT-3, respectively)³³. Additionally in NSCLC, besides the correlation between GLUT-1 and GLUT-3 with ^{18}F -FDG uptake, ^{18}F -FDG uptake is correlated with hypoxia inducible factor (HIF), vascular endothelial growth factor (VEGF) as well as the PI3K downstream target, mTOR expression levels in a recent large study¹⁴.

Consistent HIF pathway activation itself is related to GLUT-1¹³ and GLUT-3¹⁵ receptor expression in tumors. Pictilisib induced PI3K inhibition reduced the HIF pathway activation and consequently reduced GLUT-1 expression in a thyroid carcinoma xenograft model¹³.

Clinical relevance

Pictilisib does not have clinically relevant metabolic changes in normal tissues when used with chemotherapy under fasting conditions. A small, but clinically irrelevant change induced by pictilisib and chemotherapy in the hepatic ^{18}F -FDG uptake was observed. Subsequent studies should combine a normoglycemic hyperinsulinemic clamp performed during the ^{18}F -FDG tracer infusion and subsequent PET. During such a study, simultaneous assessment of both a resting and exercise muscular uptake should be performed, as skeletal muscle GLUT-4 is translocated by exercise²⁷.

Conclusion

In conclusion, this exploratory pilot study shows that in normal human tissue PI3K inhibition by pictilisib combined with chemotherapy does not influence GLUT-4 stimulation in a clinically significant way under fasting conditions.

Acknowledgements:

We are grateful for the help provided by J.H. van Snick with the logistics and execution of this study. We are tremendously grateful for all the help provided by Vikram Rao Bollineni.

This research was performed partly within the framework of CTMM, the Center for Translational Molecular Medicine, project AIRFORCE (grant 030-103). CTMM had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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